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# Measuring specific receptor binding of a PET radioligand in human brain without pharmacological blockade: the genomic plot

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**Running headline:** the genomic plot

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## Abstract

PET studies allow *in vivo* imaging of the density of brain receptor species. The PET signal, however, is the sum of the fraction of radioligand that is specifically bound to the target receptor and the non-displaceable fraction (i.e. the non-specifically bound radioligand plus the free ligand in tissue). Therefore, measuring the non-displaceable fraction, which is generally assumed to be constant across the brain, is a necessary step to obtain regional estimates of the specific fractions.

The nondisplaceable binding can be directly measured if a reference region, i.e. a region devoid of any specific binding, is available. Many receptors are however widely expressed across the brain, and a true reference region rarely available. In these cases, the nonspecific binding can be obtained after competitive pharmacological blockade, which is often contraindicated in humans.

In this work we introduce the *genomic plot* for estimating the nondisplaceable fraction using baseline scans only. The genomic plot is a transformation of the Lassen graphical method in which the brain maps of mRNA transcripts of the target receptor obtained from the Allen Brain Atlas are used as a surrogate measure of the specific binding. Thus, the genomic plot allows the calculation of the specific and nondisplaceable components of radioligand uptake without the need of pharmacological blockade.

We first assessed the statistical properties of the method with computer simulations. Then we sought ground-truth validation using human PET datasets of seven different neuroreceptor radioligands, where nonspecific fractions were either obtained separately using drug displacement or available from a true reference region. The population nondisplaceable fractions estimated by the genomic plot were very close to those measured by actual human blocking studies (mean relative difference between 2% and 7%). However, these estimates were valid only when mRNA expressions were predictive of protein levels (i.e. there were no significant post-transcriptional changes). This condition can be readily established *a priori* by assessing the correlation between PET and mRNA expression.

## INTRODUCTION

Positron emission tomography (PET) radioligands allow *in vivo* imaging of the distribution and availability of brain receptors. Amongst the criteria for a successful radioligand (Pike, 2009) a high level of specific binding is of prominent importance. The PET signal is a mixture of specific binding (i.e. the percentage of radioligand bound to the target receptor) and nondisplaceable uptake, which is the sum of nonspecific binding and free ligand in tissue (Innis et al., 2007). The ratio at equilibrium of the specifically bound radioligand to that of the nondisplaceable radioligand in tissue is the binding potential ( $BP_{ND}$ ) which reflects the affinity of the radioligand for the target and more importantly the target availability *in vivo* (Innis et al., 2007). If the nondisplaceable component is known, then the specific component, and hence  $BP_{ND}$ , can be derived from the total activity.

The nondisplaceable binding can be directly measured if a reference region, i.e. a region truly devoid of the receptors under study, is available (Lammertsma et al., 1996; Lammertsma and Hume, 1996). The radioligand concentration in this region then becomes the reference for all regions, because the nondisplaceable binding is generally assumed to be constant in the brain (Lammertsma and Hume, 1996). Many receptors are however expressed across the whole human brain, and therefore a reference region is rarely available (Turkheimer et al., 2012). In these instances, measuring the nondisplaceable fraction requires an additional scan after administration of a competitive blocking agent (Lassen et al., 1995). This can be achieved without the complete blockade of all receptors, because the parameter of interest can be derived from a simple correlation using the variation of the total binding before and after administration of the blocking drug. This approach, generally described as the Lassen plot method (Cunningham et al., 2010; Lassen et al., 1995), has however two disadvantages: 1) blocking drugs may not be safe for human use at pharmacological doses or may not be available and 2) at least two separate PET scans are necessary for each subject.

Recently, Zanotti-Fregonara et al (Zanotti-Fregonara et al., 2015) introduced a method to estimate the specific and nondisplaceable components of a PET radioligand for metabotropic glutamate receptor 1 (mGluR1), based on the correlation of the regional density of the mGluR1 gene transcript with PET measurements of the expressed protein. This analysis is a variation of the Lassen plot, which measures the linear relationship of the tracer

specific binding as a function of total distribution volume in brain. In Zanotti-Fregonara et al (Zanotti-Fregonara et al., 2015), the regional densities of the mGluR1 gene transcript in human brain were strongly and linearly correlated with regional densities (distribution volume,  $V_T$ ) of mGluR1 measured with PET. Furthermore, the extrapolated value of  $V_T$  when mGluR1 gene transcript would equal 0 (i.e., in the absence of specific binding) was a reasonably accurate measure of tracer nondisplaceable volume of distribution ( $V_{ND}$ ), based on pharmacological blockade in a monkey. This variation of the Lassen plot, which we call the “*genomic plot*”, has the significant advantage of estimating the specific and nondisplaceable components of radioligand uptake without the need for pharmacological blockade.

The purpose of this study was to assess the wider applicability of the genomic plot to estimate specific and nondisplaceable components of several PET targets, including six receptors and one enzyme. We first validated the statistical properties of the *genomic plot* with computer simulations to test its robustness against varying brain protein and mRNA patterns as well as varying mRNA-protein relations for different neurotransmitter systems. We then sought ground-truth validation using human PET datasets where estimates of the nondisplaceable fraction were available from blocking studies.

## MATERIAL AND METHODS

### Theory

In a brain PET study, the radioligand activity in the tissue is typically the sum of a specific and a nondisplaceable component (Innis et al., 2007; Lassen et al., 1995; Mintun et al., 1984). Given  $n$  regional estimates, the total volume of distribution for the  $j^{\text{th}}$  region ( $V_{T,j}$ ) is given by:

$$V_{T,j} = V_{S,j} + V_{ND} \quad \forall j = 1, \dots, n \quad (\text{Eq. 1})$$

where  $V_{S,j}$  is the regional specific distribution volumes for the  $j^{\text{th}}$  region and  $V_{ND}$  represents the nondisplaceable distribution volume. The implicit assumption of Eq. 1 is that  $V_{ND}$  is constant across all brain regions, a common and generally valid assumption in brain neuroreceptor experiments (Lammertsma et al., 1996; Lammertsma and Hume, 1996; Lassen et al., 1995).

If a close relationship exists between mRNA expression and protein concentration, the transcriptome then reflects the *in vivo* distribution of the brain protein, and therefore it can be used as a proxy of the specific binding of the radioligand. This can be written as:

$$V_{S,j} = \alpha \cdot mRNA_j \quad \forall j = 1, \dots, n \quad (\text{Eq. 2})$$

where  $mRNA_j$  represents the vector of mRNA measurements for a given gene in the  $j^{\text{th}}$  region and  $\alpha$  is the scale factor between the transcript expression (either relative or absolute) and the tracer specific binding. Notably, the use of a constant value for  $\alpha$  across the different brain regions is a strong assumption which holds only when a linear dependence exists between the mRNA expression and the correspondent protein density and when the occupancy by endogenous neurotransmitter is uniform across brain regions. When these conditions are met, by combining Eq. 1 and Eq. 2, the distribution volume of the radioligand becomes linearly related to the mRNA expression of the target as

$$V_{T,j} = \alpha \cdot mRNA_j + V_{ND} \quad \forall j = 1, \dots, n \quad (\text{Eq. 3})$$

Following the convention introduced for the Lassen plot for PET occupancy studies (Lassen et al., 1995) and given the smaller noise of PET  $V_T$  compared to mRNA data, we use  $V_T$  as the independent variable, rewriting Eq. 3 as:

$$mRNA_j = \frac{1}{\alpha} \cdot (V_{T,j} - V_{ND}) \quad \forall j = 1, \dots, n \quad (\text{Eq. 4})$$

The linear regression of these two variables ( $x = mRNA$  and  $y = V_T$ ) generates a plot where the x-axis intercept is equal to the  $V_{ND}$ . We named this method the genomic version of the Lassen plot or simply “*genomic plot*”.

### **Simulated data**

Monte-Carlo simulations were performed to assess the statistical robustness of the genomic plot (Eq. 4) in a number of realistic stochastic conditions reflecting:

- Variability of the specific binding  $V_S$  between brain regions (i.e. variability of  $V_S$  between ROIs)
- Misspecification between  $mRNA$  and  $V_S$  (i.e. variability of  $\alpha$  between ROIs) indicating lack of linearity between gene expression and measured protein levels
- Variability of the tracer binding potential (approximated by  $V_S/V_{ND}$ )

For the first condition, we simulated a spectrum of 5 cases corresponding to a  $V_S$  variability ranging from 10% (homogenous  $V_S$  distribution) to 50% (heterogenous  $V_S$  distribution). A lognormal distribution was used to avoid the generation of negative  $V_S$ .

For the second condition, we simulated 11 different scenarios of  $\alpha$  between-region variability, from 1% (no misspecification between mRNA and  $V_S$ ) to 100% (complete misspecification between mRNA and  $V_S$ ). The values were randomly generated by sampling a lognormal distribution with mean equal to 0.1 and standard deviation proportional to the variance of  $\alpha$ . This range of values for  $\alpha$  was chosen based on the results reported by Rizzo and colleagues (Rizzo et al., 2014), who showed that PET  $V_T$  estimates ( $\text{ml}/\text{cm}^3$ ) are approximately one order of magnitude lower than the linearized mRNA expression (unit less) of the correspondent target proteins. The analysis included 3 brain PET tracers ( $[^{11}\text{C}]\text{WAY100635}$ ,  $[^{11}\text{C}]\text{CUMI101}$  and  $[^{11}\text{C}]\text{DPN}$ ) with the Allen Human Brain atlas as source for the genomic information (<http://human.brain-map.org/>).

For the last condition, three different scenarios were tested: a low-binding case (mean  $V_S = 0.5 V_{ND}$ ), an intermediate-binding case (mean  $V_S = V_{ND}$ ) and a high-binding case (mean  $V_S = 2 V_{ND}$ ). Mean  $V_S$  values were used as reference to generate the regional specific bindings, and the between-region variability was defined accordingly to the first condition. These representative cases were chosen to cover the typical range of binding potential for a PET tracer (Guo et al., 2009).

$V_{ND}$  was assumed constant for all simulations ( $V_{ND} = 2 \text{ ml/cm}^3$ ), as used previously (Cunningham et al., 2010). Twelve ROIs were simulated for both PET and mRNA data, which is a typical number of regions when brain PET scan are matched with mRNA measures (Rizzo et al., 2014). For all conditions, 1,000 noisy simulations were generated by adding Gaussian distributed noise (zero mean and 5% coefficient of variation,  $CV$ ) to the total distribution volumes, independently for each simulated ROI. This procedure, as well as the noise level, were defined according to the literature (Cunningham et al., 2010). In total, 165,000 simulations (5 cases of  $V_S$  variability x 11 cases of genomic misspecification x 3 levels of binding x 1,000 simulations) were computed. A summary of the settings used for the simulations is reported in Table 1.

For each simulated scenario,  $V_{ND}$  was estimated using the genomic plot (Eq. 4), and the results compared with the correspondent simulated values. Percentage mean bias ( $\%bias$ ) and root mean square error ( $\%RMSE$ ) were used as indexes of performance:

$$\%bias = \frac{100}{N} \sum_{i=1}^N \frac{(V_{ND,i} - \bar{V}_{ND})}{\bar{V}_{ND}} \quad (\text{Eq. 5})$$

$$\%RMSE = 100 \sqrt{\frac{1}{N} \sum_{i=1}^N \left[ \frac{(V_{ND,i} - \bar{V}_{ND})}{\bar{V}_{ND}} \right]^2} \quad (\text{Eq. 6})$$

where  $N$  is number of simulations ( $N=1,000$ ),  $\bar{V}_{ND}$  is the simulated nondisplaceable distribution volume and  $V_{ND,i}$  is the  $i^{\text{th}}$  estimated nondisplaceable distribution volume. The squared Pearson's correlation coefficients ( $R^2$ ) between simulated mRNA and simulated  $V_T$  were also analysed to assess the impact of the different tested conditions on the relationship between these two quantities.

### ***In vivo positron emission tomography data***

To assess the applicability of the method to clinical data, we applied the genomic plot to the following brain PET tracers: 1) [ $^{11}\text{C}$ ]WAY100635, targeting the serotonin 5-HT<sub>1A</sub> receptor (Pike et al., 1996); 2) [ $^{11}\text{C}$ ]Ro15-4513 targeting the GABA  $\alpha 5$  receptor (Lingford-Hughes et al., 2002); 3) [ $^{11}\text{C}$ ]LY2795050 targeting the kappa opioid receptor (Zheng et al., 2013); 4) [ $^{18}\text{F}$ ]FIMX, targeting the metabotropic glutamate receptor 1 (Xu et al., 2013); 5) [ $^{11}\text{C}$ ]NOP-1A targeting the nociceptin/orphanin FQ peptide receptor (Pike et al., 2011); 6)



[<sup>11</sup>C](R)olipram, targeting the phosphodiesterase 4 enzyme (Fujita et al., 2005); 7) [<sup>11</sup>C]Raclopride, targeting the dopamine D2 receptors (Ehrin et al., 1985).

The choice of these radioligands was driven by the availability of *in vivo*  $V_{ND}$  measurements usable as the gold standard to validate the estimates obtained with the genomic plot (Table 2). Both in house data and blocking studies from literature were considered:

- [<sup>11</sup>C]WAY100635: Fifteen male healthy subjects ( $35.7 \pm 10.5$  years old) underwent a 95-min dynamic PET study in an ECAT EXACT3D (Siemens/CTI, Knoxville, TN, USA) scanner after a bolus injection of  $301 \pm 12$  MBq of [<sup>11</sup>C]WAY100635. The data were analysed as previously reported (Bose et al., 2011). The data were acquired at Imanet PET centre, London (UK).
- [<sup>11</sup>C]Ro15-4513: Data from previously reported study (Stokes et al., 2014) of four healthy male subjects ( $41.5 \pm 4.4$  years old) scanned twice were considered. All subjects underwent 90-min dynamic scan on an ECAT HR + 962 scanner (CTI/Siemens, Knoxville, TN, USA) camera after injection of  $\sim 450$  MBq of [<sup>11</sup>C]Ro15-4513. The data were acquired at the Imanet PET centre, London (UK).
- [<sup>11</sup>C]LY2795050: Sixteen healthy volunteers (24 to 56 years old; 8 males/ 8 females) underwent a 90-min dynamic PET scan on a High Resolution Research Tomograph (Siemens Medical Solutions, Knoxville, TN, USA) after intravenous administration of tracer over 1 minute by an automatic pump (injected dose:  $334 \pm 149$  MBq). Full details on PET procedures, arterial data extraction and processing are reported in (Naganawa et al., 2014). PET data were acquired at the Yale PET centre, New Haven, Connecticut (USA).
- [<sup>18</sup>F]FIMX: Twelve healthy controls ( $28 \pm 10$  years old; 4 males/8 females) underwent a 120-min dynamic PET scan after intravenous administration of  $189 \pm 3.4$  MBq of tracer. Full details on PET procedures, arterial data extraction and processing are reported in (Zanotti-Fregonara et al., 2015). PET data were acquired at the NIH PET centre, Bethesda, Maryland (USA).
- [<sup>11</sup>C]NOP-1A: Data from previously reported study (Lohith et al., 2014) of eleven healthy subjects (from 22 to 42 years old; 8 males/3 females) were considered. All the subjects underwent 120-min dynamic scan on an Advance tomograph (GE Medical

Systems, Waukesha, WI) after bolus injection of  $713 \pm 79$  MBq of  $[^{11}\text{C}]\text{NOP-1A}$ . Full details on PET protocol and data and processing are reported in (Lohith et al., 2014; Tonietto et al., 2015). PET data were acquired at the NIH PET centre, Bethesda, Maryland (USA).

- $[^{11}\text{C}](R)\text{rolipram}$ : Twelve healthy controls ( $28 \pm 11$  years old; 10 males/2 females) were included in the analysis (Zanotti-Fregonara et al., 2011). All PET images were acquired using an Advance tomograph (GE Medical Systems, Waukesha, WI, USA) after a bolus injection of  $695 \pm 152$  MBq of  $[^{11}\text{C}](R)\text{-rolipram}$ . The data were analysed as previously reported in (Rizzo et al., 2013). PET data were acquired at the NIH PET centre, Bethesda, Maryland (USA).
- $[^{11}\text{C}]\text{Raclopride}$ : Ten healthy controls ( $46.7 \pm 14.1$  years old; 8 males/2 females) were acquired using a ECAT EXACT HR++ tomograph (CTI/Siemens 966; Siemens, Knoxville, TN) after a bolus injection of 180-186 MBq (Pavese et al., 2006). PET data were acquired at the Imanet PET centre, London (UK).

For all these studies, ethical approval was granted independently by the internal ethical committees of the different institutes. Regional  $V_T$  estimates were quantified with a nonlinear weighted least square estimator applied to the 2-tissue compartmental model. For  $[^{11}\text{C}]\text{Raclopride}$ , the outcome parameter was  $BP_{ND}$ , obtained with a simplified reference tissue model using the cerebellum as the reference region. For each tracer, regional population  $V_T$  (or  $BP_{ND}$  for  $[^{11}\text{C}]\text{Raclopride}$ ) estimates were obtained by averaging results across subjects prior to genomic plot analysis (Supplementary material - Table 1-7). Notably all the datasets were acquired independently (experimental conditions change from study to study) and processed according to the goals of the clinical studies. For example, the brain segmentation was inconsistent between datasets, because for each case the region contouring was defined to best match the tracer tissue distribution. These characteristics represented the best conditions to test the flexibility, robustness and general applicability of the genomic plot.

### **mRNA data and Allen Human Brain atlas**

The mRNA transcription maps were downloaded from the Allen Human Brain atlas (ABA) (<http://www.brain-map.org>) (Hawrylycz et al., 2012). This dataset is derived from six healthy donors ( $42.5 \pm 13.4$  years old, 5 male/1 female) and contains more than 29,000 gene expression profiles sampled throughout the brain. On average,  $617 \pm 242$  samples were collected for each donor and the brain structures are sampled proportionally to their volume. For two out of the six brains, samples were collected from both hemispheres. For the remaining four, tissue samples for microarray analysis were limited to the left hemisphere.

Full details about the procedures for the tissue collection and processing, the microarray experimental design and execution, and the data quality control up to the integration of the data into the online resource are reported in the supplementary data of Hawrylycz et al (Hawrylycz et al., 2012).

The expression profiles of the genes downloaded from ABA were: *HTR1A* for [ $^{11}\text{C}$ ]WAY100635, *GABRA5* for [ $^{11}\text{C}$ ]Ro15-4513, *OPRK1* for [ $^{11}\text{C}$ ]LY2795050, *GRM1* for [ $^{18}\text{F}$ ]FIMX, *OPRL1* for [ $^{11}\text{C}$ ]NOP-1A, *PDE4A*, *PDE4B*, *PDE4C* and *PDE4D* for [ $^{11}\text{C}$ ](R)rolipram, and *DRD2* for [ $^{11}\text{C}$ ]Raclopride. For all genes and all probes, mRNA expression were downloaded at the highest spatial resolution possible (i.e., each value represented a physical tissue sample) in *log2* expression intensity. A summary of the data used for the analysis is available in the supplementary material.

### **mRNA data analysis**

The individual mRNA expressions, originally mapped in the native brain MRI space, were spatially normalised to the standard stereotaxic space (MNI/ICBM152) using the transformation matrices available from the ABA data portal. Additionally, the anatomical classifications of the sample labels were moved on the image space to obtain brain segmentation consistent with the *sample/structure/coarse* levels of resolution, as defined in ABA. Based on this match, the mRNA samples belonging to the same ROIs were averaged across different donors. The ROIs considered were: frontal, parietal, temporal and occipital lobes, cingulate gyrus, hippocampus, insula, striatum, globus pallidum, amygdala, thalamus, cerebellum and brainstem. These regions were chosen to guarantee an adequate brain coverage and a sufficiently large number of samples per region (>10 per region and per donor).

Most of the gene expressions included in the ABA database are measured with two or more probes. The presence of multiple probes for the same gene, whose expression profiles may be inconsistent, does not allow the use of the mean mRNA expression as representative of the true mean transcript expressions. Thus, it is fundamental for any type of application to select only the probe that best describes the real transcript profile and discard the remaining ones. According to (Hawrylycz et al., 2012), for each gene we selected a unique probe, representative of the gene expression. Among the available probes, the one with the least skewed distribution across donors was chosen (Figure 1).

The processing of the mRNA data was performed with MENGA (Multimodal Environment for Neuroimaging and Genomic Analysis) (Rizzo et al., 2015). This software package allows the integration of the mRNA transcript maps from ABA with any neuroimaging modality and calculates the gene vs. image cross-correlation statistics. MENGA software and manual are available at [www.fair.dei.unipd.it/software](http://www.fair.dei.unipd.it/software). All analyses, including genomic plot implementation, were performed using Matlab®2012b (The MathWorks, Inc., Natick, MA, USA) on a Windows 7 computer.

### **Genomic plot analysis**

First, mRNA data were converted from  $\log_2$  intensity into linear scale. This preliminary step is necessary to maintain a linear relationship between mRNA measures and gene transcript intensity. The values were then linearly regressed with the population  $V_T$  values from the corresponding PET tracer to determine whether the relative gene transcript was proportional to  $V_T$ . The squared Pearson's correlation coefficient ( $R^2$ ) was used to quantify the linear correlation. The  $V_{ND}$  values estimated by the *genomic plot* were then compared with the measured  $V_{ND}$  values obtained from the blocking studies available in the literature from human or nonhuman primates. For human data, the estimated  $V_{ND}$  values were directly compared to the measured ones. For primate data, interspecies differences were accounted for by correcting  $V_{ND}$  measurements for the tracer plasma free fractions of the two species ( $f_p^{humans}$  for humans and  $f_p^{monkeys}$  for monkeys, respectively). This resulted in:

$$V_{ND}^{humans} = V_{ND}^{monkeys} \cdot \frac{f_p^{humans}}{f_p^{monkeys}} \quad (\text{Eq. 7})$$

where  $V_{ND}^{monkeys}$  is the nondisplaceable distribution volume measured in blocking studies in nonhuman primate and  $V_{ND}^{humans}$  is its equivalent in humans. Equation 7 implicitly assumes that the free fraction of ligand in the nondisplaceable tissue compartment ( $f_{ND}$ ) is unchanged between species. The procedure was implemented consistently with the approach presented by Zanotti-Fregonara *et al* (Zanotti-Fregonara et al., 2015).

[ $^{11}\text{C}$ ]Raclopride is a particular case. Since this tracer targets dopamine D2 receptors, a true reference region (i.e. the cerebellum) is available. Therefore, [ $^{11}\text{C}$ ]Raclopride can be quantified with  $BP_{ND}$ , without any peripheral blood measurement. If the assumption used for  $V_T$  (i.e. mRNA predicts the protein density in vivo) holds for [ $^{11}\text{C}$ ]Raclopride, then the intercept of the regression line should cross the origin of the axes.

## RESULTS

### *Simulated data*

Figure 2 shows some representative genomic plots applied to different cases of simulated data with  $V_{ND} = 2 \text{ ml/cm}^3$  and  $BP_{ND} = 1$  (intermediate-binding case). When the genomic misspecification (i.e. the misspecification between mRNA and  $V_S$ ) is minimal ( $CV \alpha = 1\%$ , Figure 2A and C), mRNA vs.  $V_T$  correlation is very high ( $R^2 > 0.99$ ) and the bias is  $< 1\%$ . On the contrary, when misspecification is maximal ( $CV \alpha = 100\%$ ), the correlation between transcript and  $V_T$  decreases ( $R^2 = 0.40$  and  $R^2 = 0.48$ , Figure 2B and D respectively) and this affects the precision and accuracy of  $V_{ND}$  quantification. Notably, when the  $V_S$  variability between regions is maximal ( $CV V_S = 50\%$ , Figure 2C and D), the performance is better than when the specific binding is uniform across regions ( $CV V_S = 10\%$ , Figure 2A and B respectively).

These results are confirmed by the overall method performance across the different simulated scenarios (Figure 3). The correlation matrix (Figure 3A) follows a bimodal behaviour: the correlation values are high ( $R^2 > 0.8$ ) when the level of genomic misspecification is low ( $CV \alpha < 20\%$ ); with higher values for higher  $V_S$  variability. On the contrary, the lowest correlation values ( $R^2 < 0.2$ ) are found when genomic misspecification is high ( $CV \alpha > 30\%$ ). Similar to the previous cases, the higher the  $V_S$  variability between regions the better the mRNA vs.  $V_T$  correlation. These results are independent from  $V_{ND}$  and from the simulated level of specific binding.

In agreement with the correlation matrix results,  $\%bias$  is also similarly dependent on genomic misspecification and  $V_S$  variability (Figure 3B-D). The best performance is found for the lowest misspecification ( $CV \alpha = 1\%$ ) and the highest  $V_S$  variability ( $CV V_S = 50\%$ ), and the worst results are obtained in the opposite case ( $CV \alpha = 100\%$ ,  $CV V_S = 10\%$ ). Differently from the correlation analysis, the level of specific binding of the radioligand affects the overall performance: the lower the  $V_S/V_{ND}$  ratio, the lower the bias. The  $\%bias$  average (mean $\pm$ SD) is  $70\% \pm 636\%$  (median  $-2.4\%$ ) for high-binding case,  $17\% \pm 54\%$  (median  $-1.5\%$ ) for intermediate-binding case, and  $-13\% \pm 70\%$  (median  $-0.6\%$ ) for low-binding case. Consistently,  $\%RMSE$  medians range from 82% in the high-binding case to 10% in the low-binding case. The variability of  $\%bias$  highlights the high sensitivity of genomic plot performances to the

ability of mRNA to correctly predict the in vivo protein density: the reliability of the method is inversely proportional to the genomic misspecification.

This is important piece of information, as the correlation between mRNA and  $V_T$  can be used to predict the performance, here intended in terms of bias, of the genomic plot. Defining 95% of confidence interval of the estimates, simulation results suggested that the genomic plot can yield an absolute %bias<5% when  $R^2>0.4$  for low-binding scenario,  $R^2>0.6$  for intermediate-binding scenario and  $R^2>0.85$  for the high-binding scenario. Similarly, an absolute %bias< 10% could be obtained with a correlation  $R^2>0.25$  for low-binding scenario,  $R^2>0.4$  for intermediate-binding scenario and  $R^2>0.75$  for the high-binding scenario. Notably, these thresholds are valid only within the particular settings used for the simulations and need to be further validated by analysing actual measured data.

### **Performances in vivo measured datasets**

Table 2 reports a summary of the genomic results in the measured PET datasets. A variable degree of correlation between mRNA and  $V_T$  was found among the different tracers. The highest correlation was found for [ $^{18}\text{F}$ ]FIMX, [ $^{11}\text{C}$ ]WAY100635 and [ $^{11}\text{C}$ ]Ro15-4513 ( $R^2 > 0.80$ ) while a low correlation was obtained for [ $^{11}\text{C}$ ]LY2795050 ( $R^2 = 0.25$ ). For [ $^{11}\text{C}$ ]NOP1A and [ $^{11}\text{C}$ ](R)rolipram, no correlation was found ( $R^2 < 0.1$ ). Notably, the different number and type of regions used for the comparison did not appear to play a role in the correlation between PET imaging and mRNA expression. For all tracers, 9 to 12 ROIs were analysed (Table 2).

Similarly to the results obtained in simulated data, the best consistency between estimated and measured  $V_{ND}$  values was obtained for the tracers in which genomic plot showed the highest correlation between PET and mRNA expression (Figure 4). Specifically these were [ $^{11}\text{C}$ ]WAY100635 (estimated  $V_{ND} = 0.30 \text{ ml/cm}^3$  – measured  $V_{ND} 0.28\pm0.43 \text{ ml/cm}^3$ ) and [ $^{11}\text{C}$ ]Ro15-4513 (estimated  $V_{ND} = 2.14 \text{ ml/cm}^3$  – measured  $V_{ND} 2.13\pm0.80 \text{ ml/cm}^3$ ), followed by [ $^{18}\text{F}$ ]FIMX (estimated  $V_{ND}/f_p = 138 \text{ ml/cm}^3$  – measured  $V_{ND}/f_p 170 \text{ ml/cm}^3$ ).

As predicted by the lack of any correlation between mRNA and PET, inaccurate results were obtained for [ $^{11}\text{C}$ ]LY2795050 (estimated  $V_{ND} = 0.26 \text{ ml/cm}^3$  – measured  $V_{ND} 1.69\pm0.13$

ml/cm<sup>3</sup>), while for [<sup>11</sup>C]NOP1A and [<sup>11</sup>C](R)rolipram non-physiological estimates were returned by the genomic graphical analysis (Figure 5).

Finally, as predicted from theoretical considerations, the regression line between mRNA data of D2 receptors and  $BP_{ND}$  values of [<sup>11</sup>C]Raclopride crossed the origin of the axes (Figure 6).

### ***Impact of probe selection***

Since for all genes multiple probes exist on mRNA microarrays we further investigated the application of the genomic plot to [<sup>11</sup>C]WAY100635, [<sup>11</sup>C]Ro15-4513 and [<sup>11</sup>C]LY2795050 (i.e. the tracers with human blocking study available) using secondary probes that were not originally selected as representative; the result is reported in Figure 7. For each tracer most of the probes presented similar correlations with PET tracer activity and led to consistent  $V_{ND}$  estimates. Notably, these probes were also highly cross-correlated among each other (mean  $R^2 = 0.92$  for [<sup>11</sup>C]WAY100635, 0.95 for [<sup>11</sup>C]Ro15-4513 and 0.94 for [<sup>11</sup>C]LY2795050). Nevertheless, some probes did not correlate with the nominated probes nor with *in vivo* imaging ( $R^2 < 0.01$ ) and did not yield physiological  $V_{ND}$  estimates. These probes were *CUST\_15880\_PI416261804* for [<sup>11</sup>C]WAY100635 and *CUST\_495\_PI416408490* for [<sup>11</sup>C]LY2795050. Notably, both probes were characterized by a high number of low-expressed samples hence indicating that only probes with high affinity and abundant binding to the specific mRNA species should be selected for further processing.

These results were confirmed when the analysis was extended to the tracers for which only nonhuman blocking studies were available. For both [<sup>11</sup>C]NOP1A and [<sup>11</sup>C](R)rolipram, the use of secondary probes confirmed a non-significant correlation between mRNA and PET  $V_T$  ( $R^2 < 0.1$ ) and yielded negative  $V_{ND}$  estimates. Also for [<sup>18</sup>F]FIMX, the application of the genomic plot using secondary mRNA probes led to not physiological results. Notably these secondary probes were characterized by higher between-donor variability than the primary one. High correlation was found between primary and secondary probes for the DRD2 gene ( $R^2 = 0.85 \pm 0.10$ ), resulting in no significant changes in the genomic plot performance.



## DISCUSSION

In this work, we presented an original approach for estimating the specific and nondisplaceable fractions of brain PET radioligands without using pharmacologically blocked studies.

The method derives the non-specific volume of distribution  $V_{ND}$  from the linear relationship between the relative regional density of the mRNA transcripts of the target receptor (considered as proxy of the tracer specific binding) and the regional volumes of distribution of the radioligand at baseline.

The proposed approach can be considered as a genomic variant of the Lassen plot (Lassen et al., 1995), and therefore was named *genomic plot*. It is worth noting that several versions of the Lassen plot have been previously introduced, where non-specific fractions were obtained either in the absence of a drug free scan (multiple drug levels but no baseline)(Cunningham et al., 2010), with partial blocking (Owen et al., 2014), or in the presence of varying affinities across subjects due to genetic polymorphisms (Guo et al., 2014). With the genomic plot, we have introduced the use of transcript densities as a proxy for protein density and the specific volume  $V_S$ .

### **Method applicability**

Because the Allen Human Brain atlas contains the transcript maps of 29,179 human brain proteins, the genomic plot potentially has potentially wide applicability. In practice, a number of conditions affect the performance of the method. One critical assumption is related to the ability of gene transcript to predict protein density. At least three factors may potentially explain the poor correlations reported in the literature between mRNA and protein concentrations, and these may not be mutually exclusive (Maier et al., 2009). First, post-transcriptional mechanisms (e.g. transcriptional and post-transcriptional splicing, translational modifications and protein complex formations) influence the degree to which mRNA expression translates into protein expression; second, proteins may differ substantially in their *in vivo* half-lives; third, technologies may not be perfectly accurate in measuring either mRNA or protein content. In addition, limitations in *postmortem* tissue availability and quality may further degrade the quality of genomic maps and their predictive level. In general, the strength of correlation between mRNA and *in vivo* protein density varies among genes depending on

their function: it is stronger for genes related to cellular structure and lower or negligible for those related to cell development and the regulation of cellular function (Guo et al., 2008; Rizzo et al., 2014).

Other implicit assumptions for the applicability of the genomic plot regard the existence of a perfect correspondence between genomic and imaging samples a uniform occupancy of the endogenous neurotransmitter across the regions. For the first condition we developed a method that scrupulously couples brain imaging with genomic data. By taking advantage of the detailed spatial information of the ABA database, we were able to generate a univocal correspondence between imaging and mRNA samples. This procedure is implemented in the MENGA software. The heterogeneity of endogenous binding, instead, is more difficult to control and may potentially limit the applicability of the genomic plot. It is important to highlight that the characteristics of the PET population must match with those of the mRNA donors. Since the presence of neurological or psychiatric diseases can unevenly affect the tracer uptake across brain regions, we recommend the use only with healthy controls.

Nevertheless, for all tested cases, both in simulations and measured data, we found that when mRNA expressions are well correlated to the PET signal at baseline the genomic plot yields accurate and precise estimates of the tracer non-specific fraction. On the contrary, when this condition is not met, the method was ineffective and its bias directly followed the misspecification between mRNA and PET. Thus, the correlation between mRNA and  $V_T$  can be verified beforehand to predict whether the genomic plot will work for a given radioligand. Our simulations and analyses of clinical data found a mean bias lower than 10% when the correlation between mRNA and  $V_T$  ( $R^2$ ) was greater than 0.75. This threshold can be relaxed with low or intermediate-binding tracers ( $BP_{ND} \leq 1$ ), i.e. when the  $V_T$  carries more information on the  $V_{ND}$  than  $V_S$ . Similarly, both simulations and clinical analyses showed that a higher correlation can be obtained more easily when the specific binding is variable between regions rather than uniformly distributed across the brain.

### **Method performances**

The results obtained by the genomic plot on measured PET datasets in terms of accuracy matched quite well the *a priori* expectation for the different systems.  $V_{ND}$  quantification was unreliable for [ $^{11}\text{C}$ ]LY2795050, [ $^{11}\text{C}$ ]NOP1A and [ $^{11}\text{C}$ ](R)rolipram. The first two are radioligands for the opioid system. For this system, well-known post-translational

events control the production of mRNA variants and differentially modify proteins from each opioid receptor gene (Wei et al., 2004). By contrast, the target of [ $^{11}\text{C}$ ](R)rolipram is an intracellular enzyme, PDE4, which is part of the cAMP cascade. The bioactivity of this enzyme, and its affinity for [ $^{11}\text{C}$ ](R)rolipram, is modulated through phosphorylation (Fujita et al., 2005). In particular, phosphorylation is lost in non-living tissues and therefore the relationship between [ $^{11}\text{C}$ ](R)rolipram binding *in vivo* and mRNA expression post-mortem may not be preserved.

In contrast, serotonergic receptors, with the exception of the 5-HT<sub>2C</sub> sub-type (Burns et al., 1997), are not known to undergo post-translational modifications, which explains the precision of the genomic plot for [ $^{11}\text{C}$ ]WAY100635. Also, the analysis of [ $^{11}\text{C}$ ]Ro15-4513 and [ $^{18}\text{F}$ ]FIMX yielded  $V_{ND}$  estimates in agreement with those obtained by occupancy studies in human and nonhuman primate respectively. Notably, it is unknown whether the mRNA expressions of GABA  $\alpha 5$  and mGlu1 receptors can predict *in vivo* protein distribution (Berthele et al., 1999; Wisden et al., 1992), although this is likely, given the high correlation between mRNA and PET uptake ( $R^2 > 0.9$ ). Moreover, both GABA  $\alpha 5$  and mGluR1 are widespread across brain and present higher expression values in some regions (hippocampus and forebrain for GABA  $\alpha 5$ , cerebellum for mGluR1). This uneven distribution of PET uptake represents the ideal condition for using the genomic plot.

Finally, the genomic plot yielded reliable results also for [ $^{11}\text{C}$ ]Raclopride data. [ $^{11}\text{C}$ ]Raclopride, a dopamine D<sub>2</sub> radioligand, is commonly quantified using a reference region, usually the cerebellum. The outcome parameter,  $BP_{ND}$ , does not require blood sampling or correction for plasma free fraction, since both the input function and the free fraction are supposed to be equal across the different brain regions. As predicted from theoretical considerations, the intercept of the regression between mRNA and  $BP_{ND}$  values crossed the origin of the axes.

The validation of the method was limited by the blocking studies available in our centres and in the published literature. It should be noted that, compared to other studies in which only a single tracer was used for validation (e.g. Cunningham et al., 2010 or Turkheimer et al., 2007), the applicability of the methodology presented in this study was tested on multiple tracers directed to different brain systems. In particular, the use of nonhuman primate data can be justified under several aspects: 1) Nonhuman primate data

have been used to validate the Lassen plot (e.g. Cunningham et al., 2010); 2) Nonhuman primates are often used when blocking studies are not feasible in humans; 3) The application of the genomic plot to nonhuman data allows assessing the method in suboptimal conditions.

### ***Single subject applicability***

Differently from the other Lassen plot methods, the genomic plot has been tested on population averages rather than on individual data. As a result, the method yields only population  $V_{ND}$  estimates instead of single subject results. There are several reasons behind this choice, mainly related to the mRNA data characteristics. First, genomic maps and PET images cannot be derived from the same subject but belong to different populations. Second,  $V_{ND}$  values are generally quite similar among subjects of the same species, and population values are often used as measure of tracer non-specific binding performance.

Indeed, when we tried to match single PET subjects to the population mRNA, we observed significant between-subject variability (Supplementary Material – Figure 1) and percentage bias (mean $\pm$ SD = 10% $\pm$ 5%; range 1%:18%) (Supplementary Material – Figure 2). For this particular analysis, we used [ $^{11}$ C]WAY100635 because this tracer showed the best correlation with genomic expression and [ $^{11}$ C]Raclopride, for which the individual reference region was used as reference for the non-displaceable binding of each subject. Based on these results, we recommend the use of the genomic plot only at the population level.

### ***Methodological Considerations***

The genomic plot is based on a standard linear regression. Despite the simplicity of the methodology it is important to take into account the impact of axes convention or the type of estimator. Both  $V_T$  and mRNA values are noisy, and when used as independent variables the noise can bias the final estimates. In our analyses, inverting the axes (mRNA on x-axis and  $V_T$  on y-axis – corresponding to Eq. 3) led to significantly higher bias and variability for all the tested scenarios (data not shown). This is explained by the fact that regional population  $V_T$  values are generally less noisy than the correspondent mRNA data and therefore preferable as independent variables. Nevertheless, both the noise level and the variability of measurements can change depending on the radioligand and the system under study. In this case, alternative estimation methods, which take into account noise on both axes (Bekker, 1986), may be considered (e.g. likelihood estimation (Ogden, 2003) or orthogonal regression

(Varga and Szabo, 2002)). These methods, similarly to weighted estimation approaches, necessitate assumptions about the noise distributions of the measurements. Realistic assumptions are difficult to make: both  $V_T$  and mRNA are used as population averages in which it is necessary to distinguish the true inter-subject biological variability to random noise. Further investigations to solve this issue are necessary, although our analysis with the standard regression showed good performance, similarly to all the other versions of the Lassen plot (Cunningham et al., 2010; Lassen et al., 1995).

### **Probe selection**

In the Allen Human Brain database, only 30% of the genes are univocally represented by a single probe. More than 50% are described by two probes, and 19% by three or more. For the most studied proteins the number of available probes can be greater than 30 (see for example monoamine oxidase A (Zanotti-Fregonara et al., 2014)). The availability of multiple probes is not necessarily an advantage as the different specificity and sensitivity of the probes to the target can lead to inconsistent expression profiles across the brain for the same gene. Therefore, probe selection becomes a critical processing step as it heavily impacts on the final results. Notably, due to the between-probe discrepancy, probe averaging does not represent a viable strategy because the mean expression can be more inaccurate than the single probe profiles.

In this work we implemented a data-driven method for probe selection. To ensure that the method is generally applicable, no biological assumptions were used. The approach used between-donor consistency (e.g. correlation of probes expression across subjects) and abundance of probe binding to select the most representative expression profile for a given gene. Notably the comparison between mRNA and imaging can be exploited in the opposite direction, i.e. by using the PET to test mRNA expression measurements. Large numbers of *postmortem* human studies use gene transcript density as a surrogate for protein levels. When a PET radioligand exists for a protein target, the genomic plot can validate that assumption and help selecting which of the multiple transcripts should be used as a surrogate measure.

## **CONCLUSION**

The genomic variant of the Lassen plot, or *genomic plot*, allows the estimation of a PET radioligand nondisplaceable fraction in the absence of reference region and without requiring target-competition studies. The method has general applicability to any neuroreceptor PET tracer, because it relies on mRNA brain maps of the whole human genome. Nevertheless, its precision is dependent on the ability of mRNA expression to predict protein density. Therefore, the consistency between mRNA and PET should be verified *a priori*.

## **Acknowledgements**

We thank the Allen Institute for the support with the human brain atlas.

## **Disclosure**

The authors do not have any duality of interest.

**TABLE 1 – Simulation variables**

<b>Nondisplaceable binding (<math>V_{ND}</math>)</b>	
Mean (ml/cm <sup>3</sup> )	2
Type of distribution	Constant
<b>Specific binding (<math>V_s</math>)</b>	
Mean (ml/cm <sup>3</sup> )	1 (low-binding case), 2 (intermediate-binding case) and 4 (high-binding case)
CV ( $\sigma$ /mean)	10%, 20%, 30%, 40% and 50%
Type of distribution	Lognormal
<b>Scale factor between mRNA and <math>V_s</math> (<math>\alpha</math>)</b>	
Mean (ml/cm <sup>3</sup> )	0.1
CV ( $\sigma$ /mean)	1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50% and 100%
Type of distribution	Lognormal
<b>Other variables</b>	
Number of ROIs per simulated dataset	12
Number of simulations per scenario	1,000

CV: coefficient of variation;  $\sigma$ : standard deviation

**TABLE 2 – Performance of the genomic plot in the measured PET datasets**

Tracer	Target	mRNA vs. $V_T$ correlation ( $R^2$ )	N. ROIs	Estimated $V_{ND}$ ( $ml/cm^3$ )	Expected $V_{ND}$ ( $ml/cm^3$ )	Ref(s)
$[^{11}C]WAY100635$	serotonin 5-HT1A receptor	0.91	12 <sup>a</sup>	0.30±0.02	0.28±0.43 <sup>†</sup>	(Cunningham et al., 2010)
$[^{11}C]Ro15-4513$	GABA $\alpha 5$ receptor	0.80	10 <sup>b</sup>	2.14±0.24	2.13±0.80 <sup>†</sup>	(Myers et al., 2015)
$[^{11}C]LY2795050$	kappa opioid receptor	0.25	11 <sup>c</sup>	0.26±3.43	1.69±0.13 <sup>†</sup>	(Naganawa et al., 2014)
$[^{18}F]FIMX$	metabotropic glutamate receptor 1	0.99	11 <sup>d</sup>	0.47±0.02 ( $V_{ND}/f_p^{humans} = 138$ )	0.58±0.03 <sup>‡</sup> ( $V_{ND}/f_p^{monkeys} = 170$ )	(Zanotti-Fregonara et al., 2015)
$[^{11}C]NOP-1A$	nociceptin/orphanin FQ peptide receptor	<0.01	9 <sup>e</sup>	n.a.	7.08 ± 0.47 <sup>  </sup>	(Kimura et al., 2011)
$[^{11}C](R)rolipram$	phosphodiesterase 4 enzyme	<0.01	12 <sup>a</sup>	n.a.	0.60 ± 0.12 <sup>‡</sup> ( $V_{ND}/f_p^{monkeys} = 9.63$ )	(Unpublished data from the NIH)

$V_T$ : total distribution volume;  $V_{ND}$ : nondisplaceable distribution volume  $f_p$ : free plasma fraction; n.a.: not available.

#### REGION SEGMENTATIONS:

<sup>a</sup> Frontal lobe, Parietal lobe, Temporal lobe, Occipital lobe, Cingulate gyrus, Insula, Striatum, Globus pallidum, Basal forebrain, Amygdala, Thalamus, Cerebellum

<sup>b</sup> Frontal lobe, Parietal lobe, Temporal lobe, Occipital lobe, Cingulate gyrus, Hippocampus, Insula, Striatum, Thalamus, Cerebellum

<sup>c</sup> Frontal lobe, Temporal lobe, Occipital lobe, Cingulate gyrus, Hippocampus, Insula, Striatum, Globus pallidum, Amygdala, Thalamus, Cerebellum

<sup>d</sup> Frontal lobe, Parietal lobe, Temporal lobe, Occipital lobe, Cingulate gyrus, Hippocampus, Insula, Striatum, Amygdala, Thalamus, Cerebellum

<sup>e</sup> Frontal lobe, Parietal lobe, Temporal lobe, Occipital lobe, Cingulate gyrus, Striatum, Thalamus, Cerebellum, Brainstem

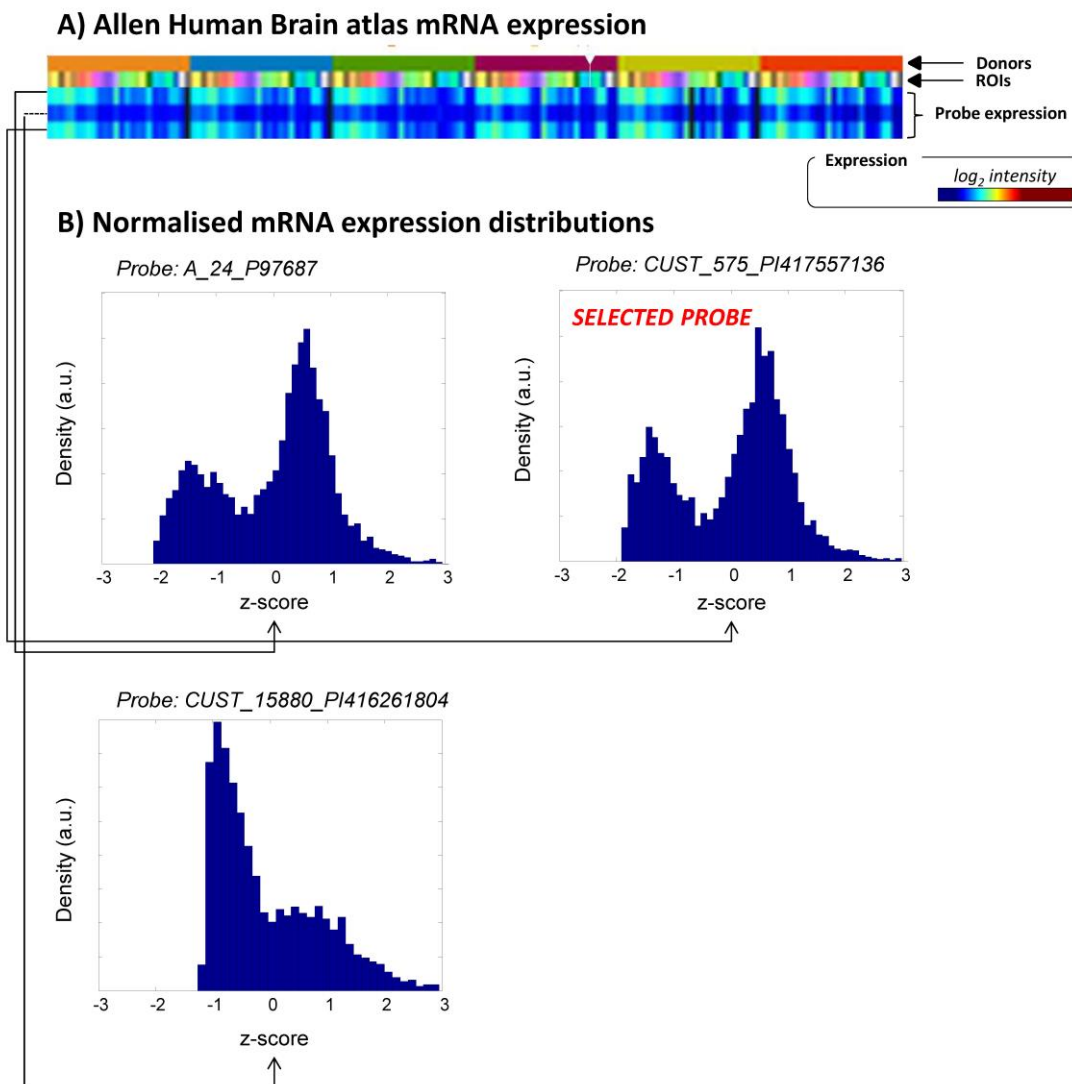
#### TYPE OF BLOCKING

<sup>†</sup> Blocking study in humans; <sup>‡</sup> Blocking study in monkeys corrected for  $f_p$  interspecies differences; <sup>||</sup> Blocking study in monkeys NOT corrected for  $f_p$  interspecies differences

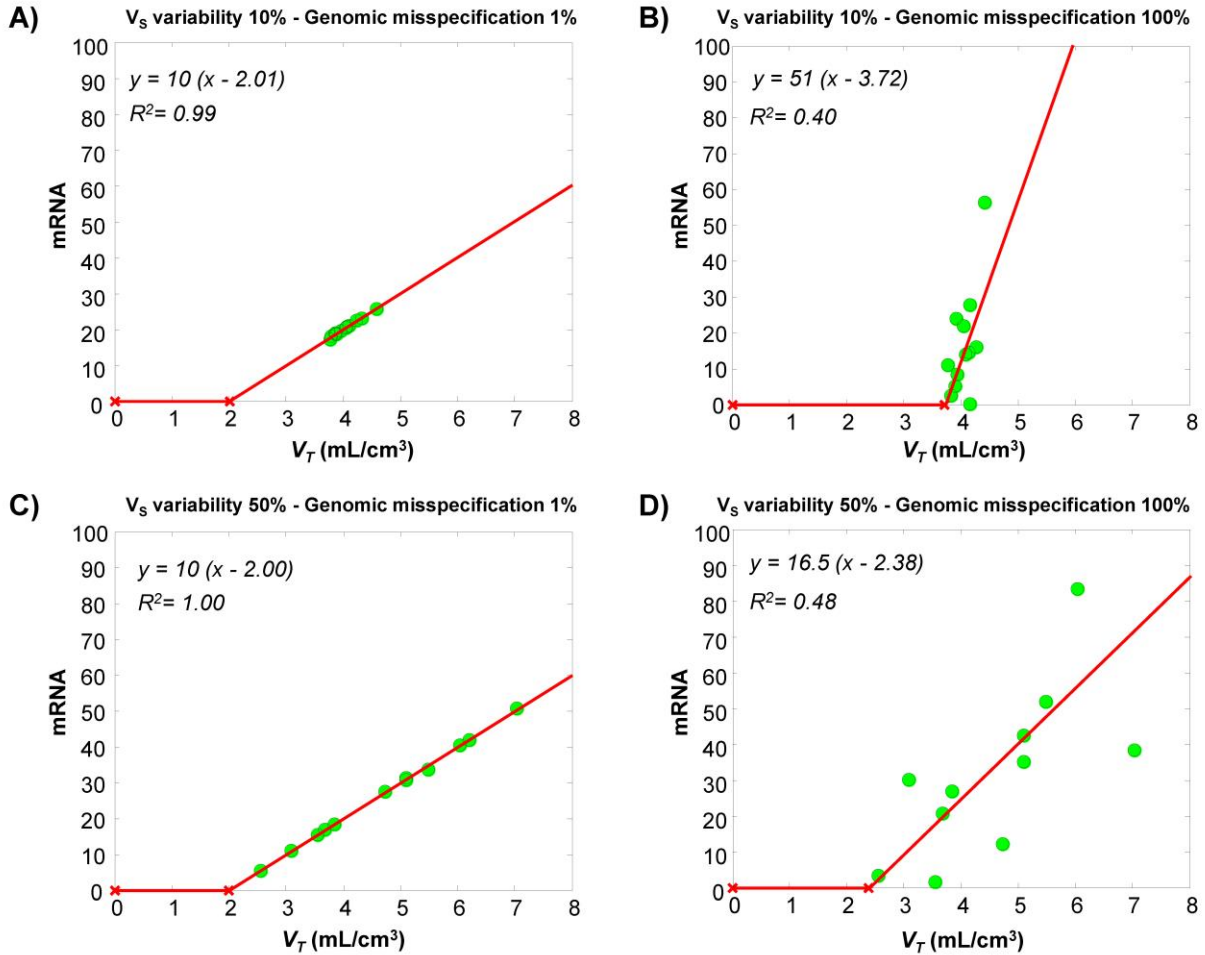


## FIGURES

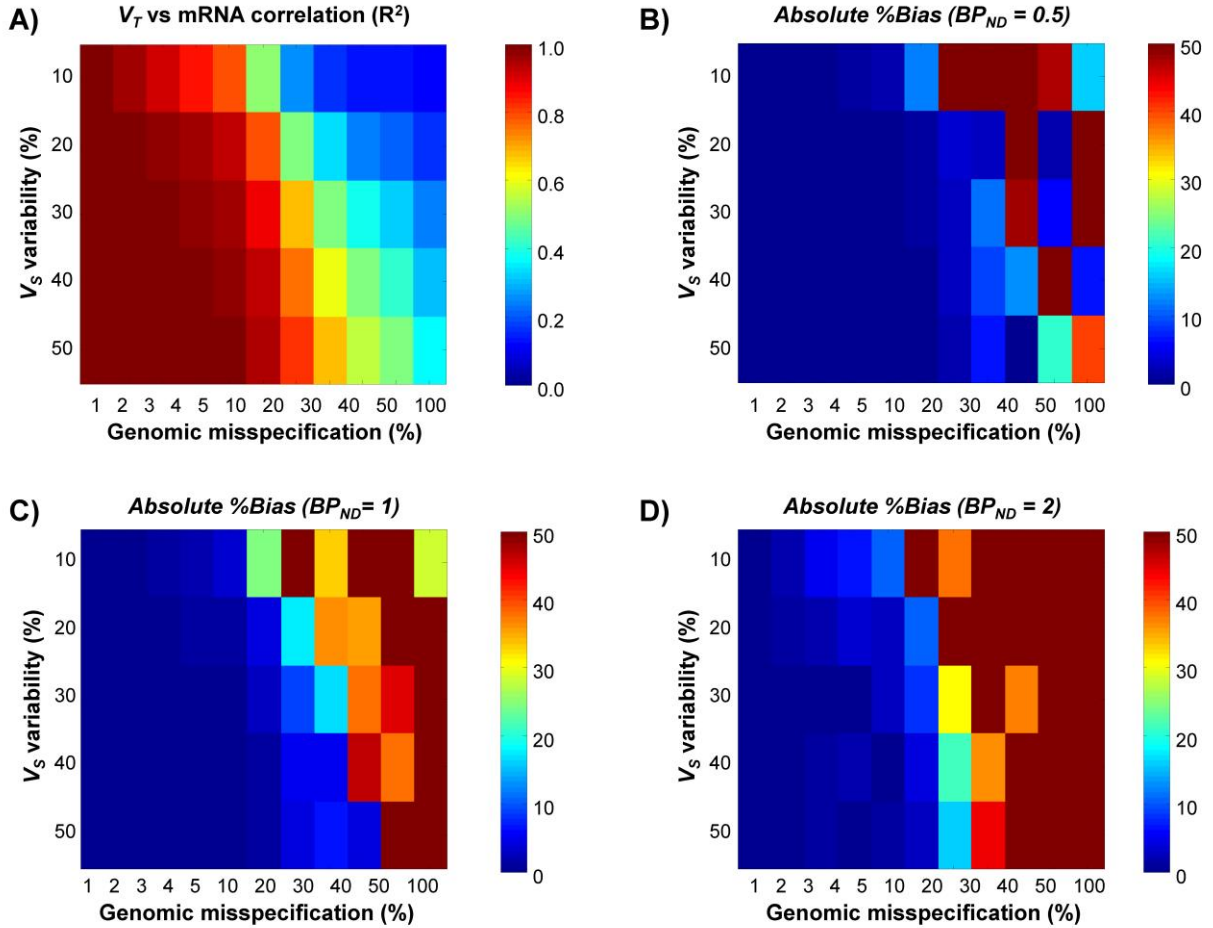
**Figure 1 – Example of probe selection for the 5TH1A receptor.** A) Probe expression profiles as derived from the Allen Human Brain atlas (<http://human.brain-map.org/>) searching for *HTR1A* gene (setting: course level, log2 intensity, 6 donors). B) Normalised mRNA expression distributions (z-score) for the 3 probes of the HTR1A gene. For each probe the data from all the donors are reported. The probe of choice was CUST\_575\_PI417557136. The selection was done by comparing the normalised distributions and by selecting the one with the maximum correspondence to the highest z-score value (For further information please refer to MENGA's software manual - [www.fair.dei.unipd.it/software](http://www.fair.dei.unipd.it/software)).



**Figure 2 – Examples of simulated data.** Representative genomic plots in different simulated conditions: 10% of  $V_S$  variability and 1% of genomic misspecification (A); 10% of  $V_S$  variability and 100% of genomic misspecification (B); 50% of  $V_S$  variability and 1% of genomic misspecification (C); 50% of  $V_S$  variability and 100% of genomic misspecification (D). In all the cases  $V_{ND}$  and mean  $V_S$  are equal to 2 ml/cm<sup>3</sup> (corresponding to intermediate-binding simulated scenario).

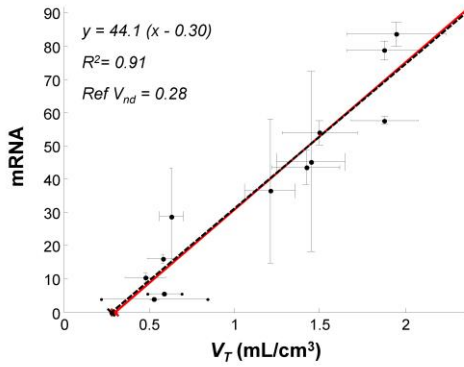


**Figure 3 – Performance of the genomic plot in simulated data.** A)  $V_T$  vs. mRNA mean correlation ( $R^2$ ) as function of  $V_S$  variability and genomic misspecification. B-D) Absolute %bias as function of  $V_S$  variability and genomic misspecification in low-binding scenario ( $BP_{ND} = 0.5$ ), intermediate-binding scenario ( $BP_{ND} = 1$ ) and high-binding scenario ( $BP_{ND} = 2$ ) respectively.

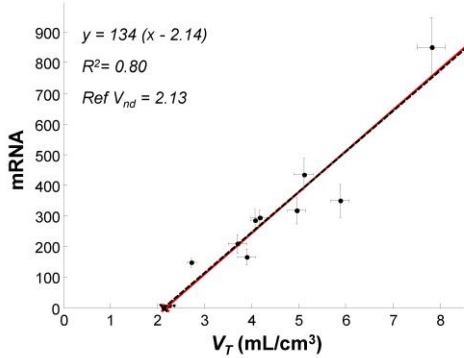


**Figure 4 – Genomic plot in PET measured data: *successful cases*.** A) [ $^{11}\text{C}$ ]WAY100635 (15 subjects), B) [ $^{11}\text{C}$ ]Ro15-4513 (4 subjects) and C) [ $^{18}\text{F}$ ]FIMX (12 subjects). Data points (●) indicate regional population values. Both  $V_T$  (x-axis) and mRNA (y-axis) standard errors are reported. Red lines refer to genomic plot regressions. Black dashed lines refer to the data regression with x-intercepts forced to the reference  $V_{ND}$  values, i.e. the  $V_{ND}$  values as derived from independent blocking studies. Please note that with [ $^{18}\text{F}$ ]FIMX the correlation is driven by a very high uptake region (i.e. cerebellum). When this is removed from the plot, the correlation is still significant ( $R^2 = 0.65$ ).

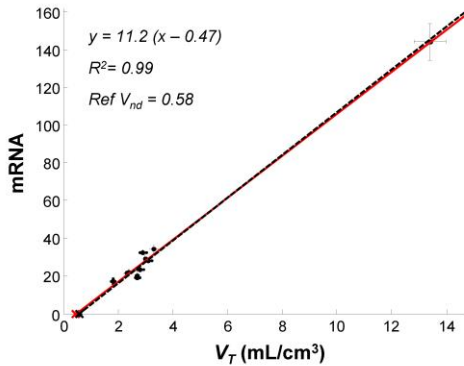
**A) [ $^{11}\text{C}$ ]WAY100635**



**B) [ $^{11}\text{C}$ ]Ro15-4513**

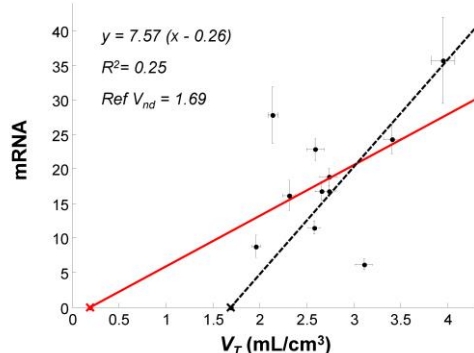


**C) [ $^{18}\text{F}$ ]FIMX**

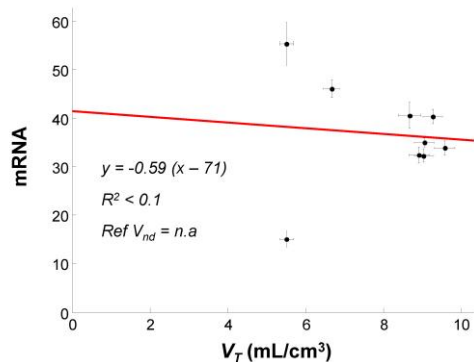


**Figure 5 – Genomic plot in PET measured data: unsuccessful cases.** A) [ $^{11}\text{C}$ ]LY2795050 (16 subjects), B) [ $^{11}\text{C}$ ]NOP1A (11 subjects) and C) [ $^{11}\text{C}$ ](R)rolipram (12 subjects). Data points (●) indicate regional population values. Both  $V_T$  (x-axis) and mRNA (y-axis) standard errors are reported. Red lines refer to genomic plot regressions. Black dashed lines refer to the data regression with x-intercepts forced to the reference  $V_{ND}$  values, i.e. the  $V_{ND}$  values as derived from independent blocking studies.

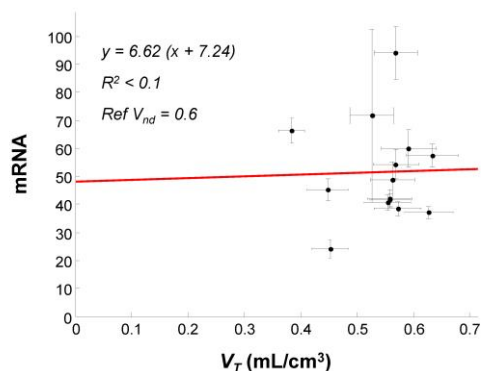
**A) [ $^{11}\text{C}$ ]LY2795050**



**B) [ $^{11}\text{C}$ ] NOP-1A**

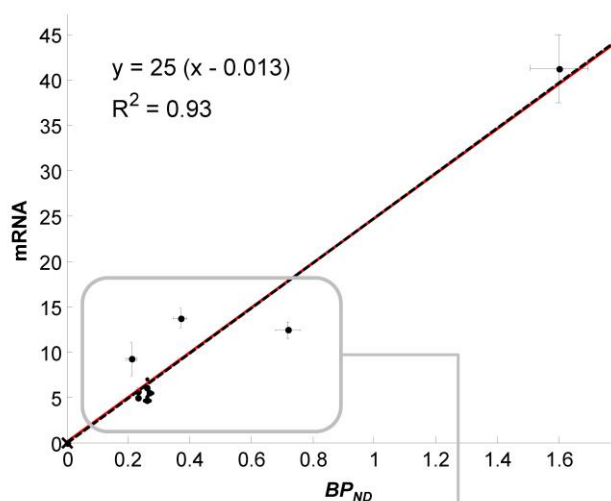


**C) [ $^{11}\text{C}$ ](R)rolipram**

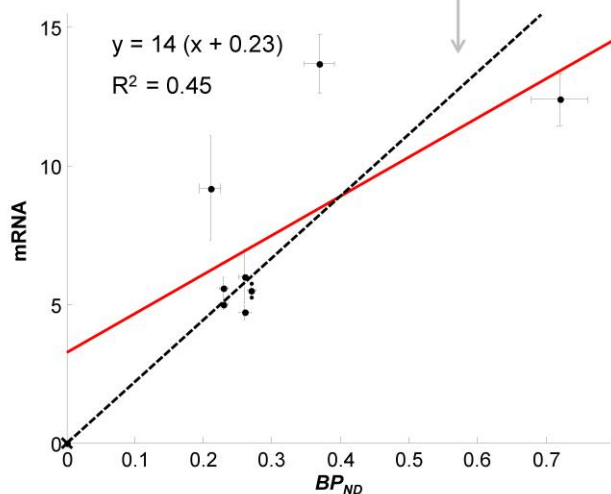


**Figure 6 – Genomic plot application to [ $^{11}\text{C}$ ]Raclopride brain PET data.** The figure shows the regression between mRNA D2 receptor expressions and [ $^{11}\text{C}$ ]Raclopride  $BP_{ND}$  estimates as computed with the simplified reference tissue model, using the cerebellum as reference region (10 subjects). Analyses were performed in the following region of interest: frontal lobe, parietal lobe, temporal lobe, occipital lobe, cingulate gyrus, hippocampus, insula, striatum and thalamus (A). When the striatum is removed the correlation dropped and with it the performance of the method (B). Both  $BP_{ND}$  (x-axis) and mRNA (y-axis) standard errors are reported. Red lines refer to genomic plot regressions. Black dashed lines refer to the data regression with x-intercepts forced to the origin.

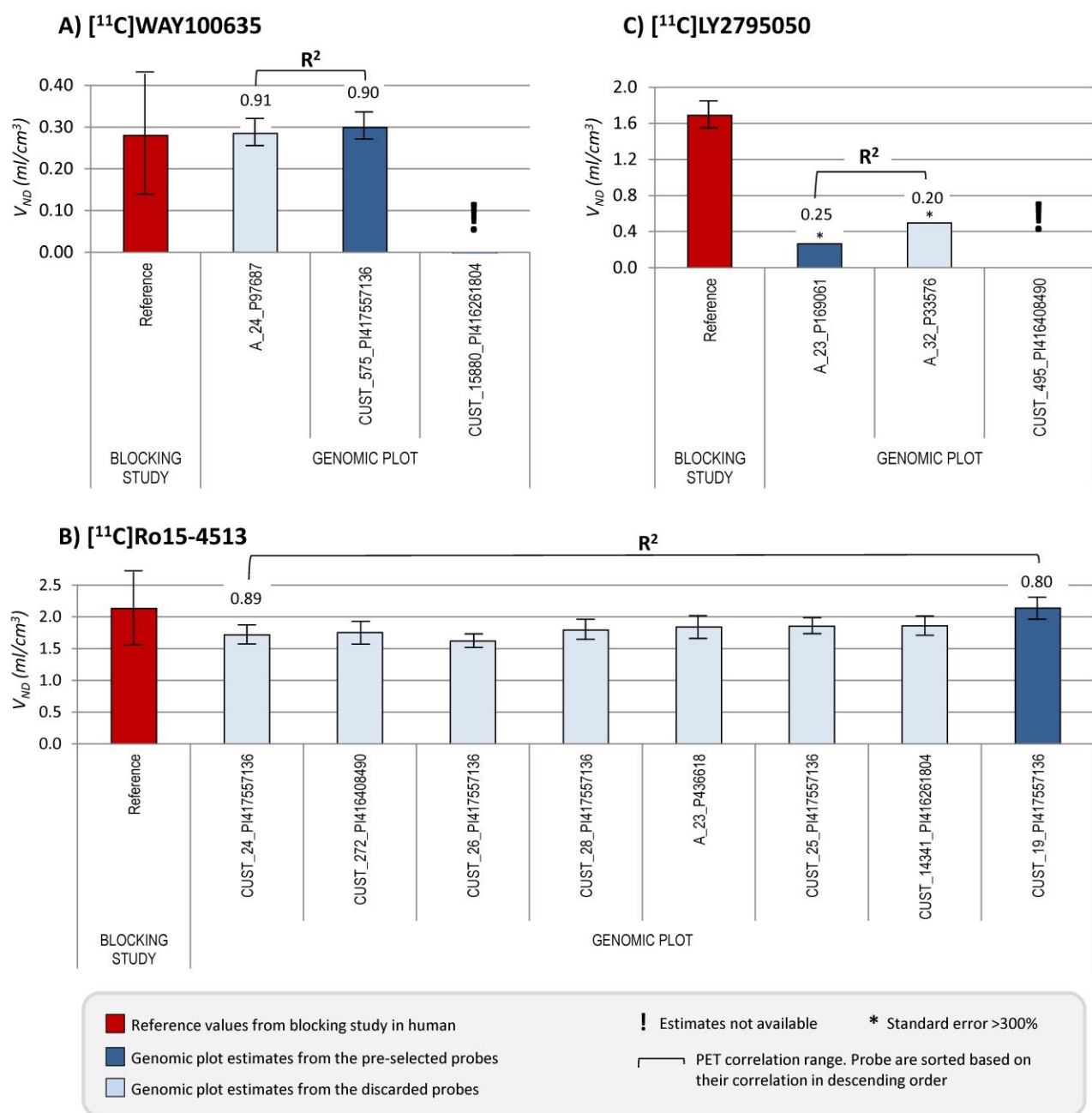
**A) [ $^{11}\text{C}$ ]Raclopride with Striatum**



**B) [ $^{11}\text{C}$ ]Raclopride without Striatum**



**Figure 7 – Impact of probe selection on [ $^{11}\text{C}$ ]WAY100635 (A), [ $^{11}\text{C}$ ]Ro15-4513 (B) and [ $^{11}\text{C}$ ]LY2795050 (C) analysis.** Reference  $V_{ND}$  estimates as obtained from blocking studies (red bars) are compared with those obtained from genomic plot applications (blue bars). For each tracer all the probes available from the Allen Human Brain database were tested. Dark blue bars refer to  $V_{ND}$  estimates as obtained from the probes selected *a priori* from the database. Light blue bars refer to  $V_{ND}$  estimates derived by the probes discarded from the initial selection. For each probe the  $V_{ND}$  standard error and the correlation with PET tracer uptake are also reported (squared Pearson's correlation coefficient,  $R^2$ ). Probes are sorted in descending order based on their correlation values with PET.



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# Measuring specific receptor binding of a PET radioligand in human brain without pharmacological blockade: the genomic plot

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## Supplementary Material

**Supplementary Table 1 – [<sup>11</sup>C]WAY100635 data**

ROIs	$V_T$ (ml/cm <sup>3</sup> )			mRNA (unitless)		
Frontal lobe	1.50	±	0.85	53.9	±	8.7
Parietal lobe	1.42	±	0.77	43.4	±	12.4
Temporal lobe	1.88	±	0.85	78.7	±	7.2
Occipital lobe	1.21	±	0.58	36.5	±	53.3
Cingulate gyrus	1.88	±	0.77	57.6	±	3.0
Hippocampus	1.81	±	1.05	n.a.	±	n.a.
Insula	1.95	±	1.12	83.6	±	9.0
Striatum	0.59	±	0.39	5.6	±	0.6
Globus pallidus	0.48	±	0.46	10.5	±	3.3
Basal forebrain	0.63	±	0.27	28.8	±	35.2
Clastrum	n.a.	±	n.a.	n.a.	±	n.a.
Amygdala	1.45	±	0.77	45.2	±	66.4
Thalamus	0.58	±	0.35	16.0	±	3.4
Cerebellum	0.53	±	0.58	3.8	±	0.2
Brainstem	0.52	±	0.39	n.a.	±	n.a.

Distribution volume estimates ( $V_T$ ) refer to a population of 15 healthy subjects (Bose et al., 2011). Genomic data (mRNA) are derived from the Allen human brain atlas (query: HTR1A). For each region the average values and the standard deviations across subjects are reported.

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**Supplementary Table 2 – [<sup>11</sup>C]Ro15-4513 data**

ROIs	$V_T$ (ml/cm <sup>3</sup> )			mRNA (unitless)		
Frontal lobe	4.07	±	0.44	285	±	71
Parietal lobe	3.70	±	0.51	208	±	57
Temporal lobe	4.96	±	0.53	317	±	81
Occipital lobe	3.90	±	0.48	165	±	51
Cingulate gyrus	5.89	±	0.52	349	±	104
Hippocampus	7.82	±	0.85	852	±	76
Insula	5.11	±	0.54	436	±	182
Striatum	4.18	±	0.51	293	±	95
Globus pallidus	n.a.	±	n.a.	27	±	49
Basal forebrain	n.a.	±	n.a.	204	±	9
Clastrum	n.a.	±	n.a.	818	±	84
Amygdala	7.61	±	0.87	n.a.	±	n.a.
Thalamus	2.73	±	0.2	148	±	36
Cerebellum	n.a.	±	n.a.	7	±	30
Brainstem	n.a.	±	n.a.	n.a.	±	n.a.

Distribution volume estimates ( $V_T$ ) refer to a population of 4 healthy subjects (Stokes et al., 2014). Genomic data (mRNA) are derived from the Allen human brain atlas (query: GABRA5). For each region the average values and the standard deviations across subjects are reported.

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**Supplementary Table 3 – [<sup>11</sup>C]LY2795050 data**

ROIs	$V_T$ (ml/cm <sup>3</sup> )			mRNA (unitless)		
Frontal lobe	2.66	±	0.29	16.8	±	3.1
Parietal lobe	n.a.	±	n.a.	13.7	±	2.7
Temporal lobe	2.74	±	0.30	16.9	±	1.9
Occipital lobe	2.58	±	0.26	11.5	±	2.5
Cingulate gyrus	2.74	±	0.38	18.8	±	3.1
Hippocampus	2.31	±	0.28	16.2	±	5.5
Insula	3.41	±	0.34	24.4	±	5.2
Striatum	2.60	±	0.36	22.9	±	3.9
Globus pallidus	3.11	±	0.37	6.1	±	2.0
Basal forebrain	n.a.	±	n.a.	20.6	±	8.0
Clastrum	n.a.	±	n.a.	76.6	±	19.0
Amygdala	3.95	±	0.51	35.8	±	15.3
Thalamus	2.14	±	0.21	27.8	±	10.2
Cerebellum	1.96	±	0.18	8.8	±	4.0
Brainstem	n.a.	±	n.a.	17.8	±	5.0

Distribution volume estimates ( $V_T$ ) refer to a population of 16 healthy subjects (Naganawa et al., 2014). Genomic data (mRNA) are derived from the Allen human brain atlas (query: OPRK1). For each region the average values and the standard deviations across subjects are reported.

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**Supplementary Table 4 – [<sup>11</sup>C]FIMX data**

ROIs	$V_T$ (ml/cm <sup>3</sup> )			mRNA (unitless)		
Frontal lobe	2.4	±	0.31	22.3	±	3.1
Parietal lobe	2.3	±	0.28	21.4	±	3.3
Temporal lobe	3.1	±	0.37	28.3	±	3.2
Occipital lobe	2.7	±	0.46	19.3	±	3.4
Cingulate gyrus	2.8	±	0.41	23.5	±	3.5
Hippocampus	2.9	±	0.39	32.6	±	3.7
Insula	3.0	±	0.41	29.1	±	4.8
Striatum	1.8	±	0.21	17.4	±	3.4
Globus pallidus	n.a.	±	n.a.	22.0	±	5.3
Basal forebrain	n.a.	±	n.a.	21.5	±	3.5
Clastrum	n.a.	±	n.a.	17.9	±	9.1
Amygdala	2.7	±	0.37	20.4	±	4.7
Thalamus	3.3	±	0.50	34.3	±	5.2
Cerebellum	13.4	±	1.90	144.1	±	24.0
Brainstem	n.a.	±	n.a.	14.2	±	2.4

Distribution volume estimates ( $V_T$ ) refer to a population of 12 healthy subjects (Zanotti-Fregonara et al., 2015). Genomic data (mRNA) are derived from the Allen human brain atlas (query: GMR1). For each region the average values and the standard deviations across subjects are reported.

## References

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**Supplementary Table 5 – [<sup>11</sup>C]NOP-1A data**

ROIs	$V_T$ (ml/cm <sup>3</sup> )			mRNA (unitless)		
Frontal lobe	9.0	±	0.83	32.4	±	3.3
Parietal lobe	9.1	±	0.87	35.0	±	2.2
Temporal lobe	9.6	±	0.85	34.0	±	3.7
Occipital lobe	9.3	±	0.89	40.4	±	3.7
Cingulate gyrus	8.9	±	0.88	32.5	±	4.1
Hippocampus	n.a.	±	n.a.	47.9	±	11.4
Insula	n.a.	±	n.a.	30.1	±	5.8
Striatum	8.7	±	0.94	40.7	±	6.6
Globus pallidus	n.a.	±	n.a.	21.4	±	3.8
Basal forebrain	n.a.	±	n.a.	41.3	±	5.6
Clastrum	n.a.	±	n.a.	53.9	±	14.4
Amygdala	n.a.	±	n.a.	54.6	±	9.1
Thalamus	5.5	±	0.57	55.5	±	10.9
Cerebellum	5.5	±	0.57	15.1	±	3.9
Brainstem	6.7	±	0.67	46.2	±	4.6

Distribution volume estimates ( $V_T$ ) refer to a population of 11 healthy subjects (Lohith et al., 2014). Genomic data (mRNA) are derived from the Allen human brain atlas (query: OPRL1). For each region the average values and the standard deviations across subjects are reported.

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**Supplementary Table 6 – [<sup>11</sup>C](R)rolipram data**

ROIs	$V_T$ (ml/cm <sup>3</sup> )			mRNA (unitless)		
Frontal lobe	0.53	±	0.13	71.7	±	75.6
Parietal lobe	0.56	±	0.14	42.1	±	7.7
Temporal lobe	0.56	±	0.13	41.7	±	5.0
Occipital lobe	0.45	±	0.13	45.2	±	9.5
Cingulate gyrus	0.57	±	0.14	38.5	±	6.4
Hippocampus	0.55	±	0.15	n.a.	±	n.a.
Insula	0.63	±	0.15	37.1	±	5.1
Striatum	0.63	±	0.16	57.5	±	10.2
Globus pallidus	0.57	±	0.13	94.1	±	23.2
Basal forebrain	0.59	±	0.17	60.0	±	16.5
Clastrum	n.a.	±	n.a.	54.5	±	15.2
Amygdala	0.56	±	0.14	48.7	±	16.0
Thalamus	0.57	±	0.14	54.3	±	12.9
Cerebellum	0.45	±	0.11	24.2	±	7.8
Brainstem	0.38	±	0.08	n.a.	±	n.a.

Distribution volume estimates ( $V_T$ ) refer to a population of 12 healthy subjects (Zanotti-Fregonara et al., 2011). Genomic data (mRNA) are derived from the Allen human brain atlas (query: PDE4). For each region the average values and the standard deviations across subjects are reported.

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**Supplementary Table 7 – [<sup>11</sup>C]Raclopride**

ROIs	$BP_{ND}$ (unitless)			mRNA (unitless)		
Frontal lobe	0.26	±	0.03	4.7	±	0.6
Parietal lobe	0.23	±	0.02	5.0	±	0.5
Temporal lobe	0.27	±	0.02	5.5	±	0.6
Occipital lobe	0.23	±	0.02	5.6	±	1.0
Cingulate gyrus	0.26	±	0.03	6.0	±	2.4
Hippocampus	0.21	±	0.05	9.2	±	4.6
Insula	0.34	±	0.06	5.3	±	1.0
Striatum	1.60	±	0.30	41.3	±	9.2
Globus pallidus	n.a.	±	n.a.	n.a.	±	n.a.
Basal forebrain	0.72	±	0.13	12.4	±	2.3
Clastrum	n.a.	±	n.a.	11.3	±	3.1
Amygdala	n.a.	±	n.a.	9.5	±	2.1
Thalamus	0.37	±	0.07	13.7	±	2.6
Cerebellum	Reference region – discarded from the analysis					
Brainstem	n.a.	±	n.a.	15.3	±	2.5.

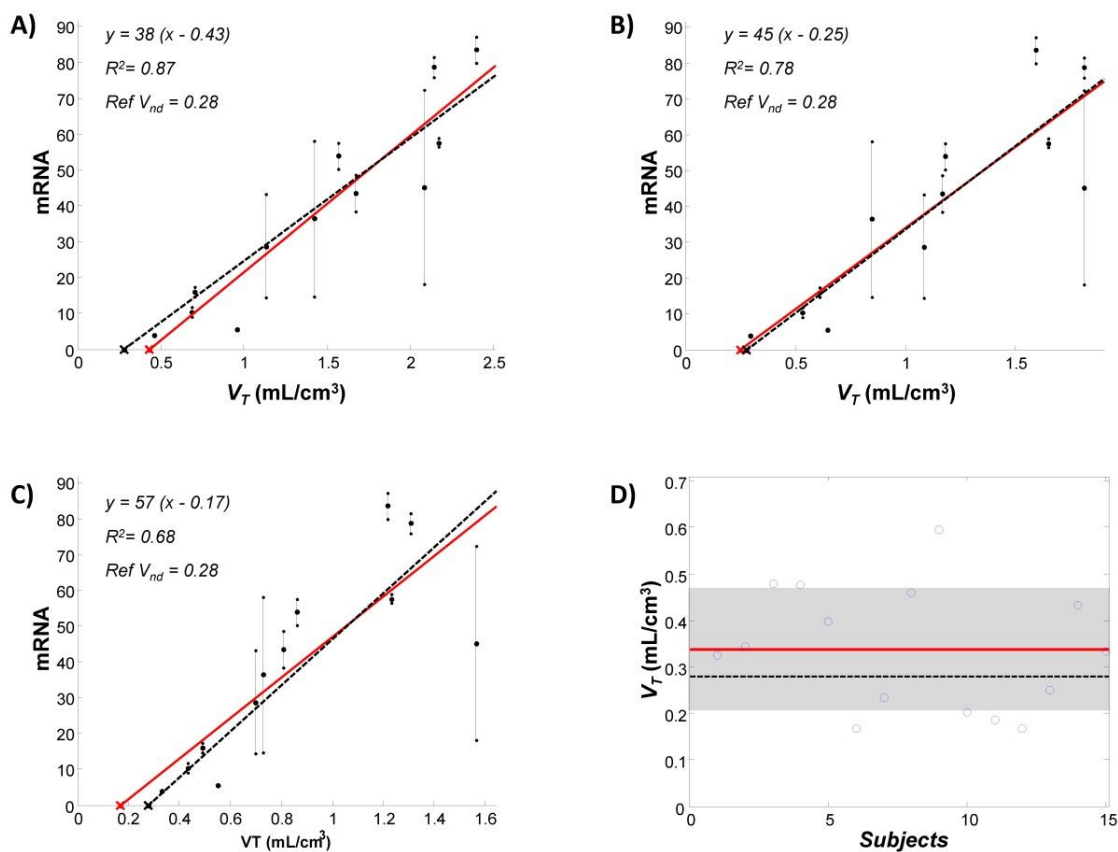
Binding potential ( $BP_{ND}$ ) refer to a population of 10 healthy subjects (Pavese et al., 2006). Genomic data (mRNA) are derived from the Allen human brain atlas (query: DRD2). For each region the average values and the standard deviations across subjects are reported.

### References

Pavese, N., Gerhard, A., Tai, Y., Ho, A., Turkheimer, F., Barker, R., Brooks, D., Piccini, P., 2006. Microglial activation correlates with severity in Huntington disease A clinical and PET study. *Neurology* 66, 1638-1643.

### Supplementary Figure 1 – Single subject analysis with [ $^{11}\text{C}$ ]WAY100635 PET data

Panels A-C show some representative single-subject genomic plot applications. Both  $V_T$  (x-axis) and mRNA (y-axis) standard errors are reported. Red lines refer to genomic plot regressions. Black dashed lines refer to the data regression with x-intercepts forced to the population  $V_{ND}$  reference value. Panel D shows the distribution of  $V_{ND}$  estimates obtained with the single subject analysis. Population  $V_{ND}$  reference estimate (red line) and its standard deviation (grey area) are compared with single subject  $V_{ND}$  estimates (blue circles). Population average of  $V_{ND}$  individual estimates (dashed black line) is also reported.



## Supplementary Figure 2 – Single subject analysis with [ $^{11}\text{C}$ ]Raclopride PET data

Panels A-C show some representative single-subject genomic plot applications. Both  $BP_{ND}$  (x-axis) and mRNA (y-axis) standard errors are reported. Red lines refer to genomic plot regressions. Black dashed lines refer to the data regression with x-intercepts forced to the origin. Panel D shows the  $V_{ND}$  absolute biases, defined as the relative difference between the individual genomic plot estimates and the single-subject reference values (as defined by the reference region).

